UNIVERSITI PUTRA MALAYSIA

MIXED MICROALGAE CULTIVATED IN OUTDOOR OPEN POND SYSTEM UNDER VARIABLE WEATHER CONDITIONS

TUNKU AINNA BINTI TUNKU AZMIR SHAH

FS 2016 57

MIXED MICROALGAE CULTIVATED IN OUTDOOR OPEN POND SYSTEM UNDER VARIABLE WEATHER CONDITIONS

By

TUNKU AINNA BINTI TUNKU AZMIR SHAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

June 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

MIXED MICROALGAE CULTIVATED IN OUTDOOR OPEN POND SYSTEM UNDER VARIABLE WEATHER CONDITIONS

By

TUNKU AINNA BINTI TUNKU AZMIR SHAH

June 2016

Chairman : Hishamuddin Bin Omar, PhD
Faculty : Science

Microalgae are known for its ability to be potential biofuel substituting fossil fuel and as a biomitigation strategy to eliminate excessive amount of carbon dioxide within earth's atmosphere. Mixed microalgae biodiversity acts as a synthetic ecosystem. Variant species mutually exploits complementary metabolic activities which are used for algal growth, survival and reproduction. This study utilizes mixed microalgae growth (eutrophication) from tilapia fish pond and benefiting the algae for its latent potentials; as a promising alternative for not only fuel, but also animal feed and food supplement. The objectives of this research are; to determine the productivity and the Specific Growth Rate (SGR), to examine the algal succession and species identification, to quantify the biochemical composition (%) and toxicity of the harvested mixed microalgae throughout three different weather conditions during the cultivation process.

Productivity and Specific Growth Rate of the mixed microalgae was deliberated via three independent analysis; optical density (680nm), biomass dry weight, and chlorophyll *a*. The estimation of species concentration (%) was using the Neubauer counting slide and visualized via Olympus BH2 light microscope at 400× and 1000× magnification. Most diatoms and dinoflagellates were identified to species level with the aid of algal taxonomy reference book. Biochemical quantification that was conducted were; soluble protein, Bradford method; carbohydrate, Soluble carbohydrate method; and lipid, modified Bligh and Dyer method. The toxicity analysis was performed by exposing Brine Shrimp (*Artemia nauplii*) to extracted mixed microalgae as toxicant.

An optimization of the algal concentration (mixed microalgae/tap water) % v/v was conducted as preliminary study. Due to good increment in algal growth, two of the highest productivity and SGR; 50 % v/v and 75 % v/v was then cultured in three weather conditions; rainy weather; culture faced rainfall throughout culture period, mixed weather; culture only faced rainfall during several days of culture period, and sunny weather; culture did not face rainfall throughout culture period. The highest productivity; 0.04 ± 0.00 g L^{-1} d^{-1} and Specific Growth Rate; 0.21 ± 0.02 µ d^{-1} documented was from Treatment 2; 75% v/v algal concentration cultured during sunny weather condition. Throughout the 30 days of cultivation (3 complete weather conditions); 4 species were identified during rainy season, 8 species during mixed weather condition and 12 species during sunny weather conditions. Several species did exist predominantly during all three weather conditions, thus, bringing the total number of 13 identified species. Biochemical analyses quantified the highest protein content (%); 46.88±0.23, carbohydrate content (%); 31.36±0.21 and lipid content (%); 29.41±0.24 which was all cultured via Treatment 2 during sunny weather condition. The toxicity analysis proved that the cultured mixed microalgae are suitable for consumption with the LD_{50} value; 59.33 % when it was exposed to 30 mg L^{-1} of extracted mixed microalgae which is considered non toxic. Sufficient sunlight irradiation, optimum culture conditions, combined with effective ambient state will generate high algal productivity with infinite potentials. However, the insights on mixed microalgae cultivation and other factors influencing the process could be conducted in future to examine the efficiency of the culture at various treatment levels.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

MIKROALGA CAMPURAN YANG DIKULTUR DALAM SISTEM KOLAM TERBUKA DALAM PELBAGAI JENIS CUACA

Oleh

TUNKU AINNA BINTI TUNKU AZMIR SHAH

Jun 2016

Pengerusi : Hishamuddin Bin Omar, PhD
Fakulti : Sains

Mikroalga terkenal dengan keupayaan untuk menjadi biofuel, menggantikan bahan api fosil dan juga digunakan dalam stratergi untuk biomitigasi lebihan karbon dioksida yang terkandung dalam atmosfera bumi. Kepelbagaian jenis spesis dalam mikroalga campuran bertindak sebagai ekosistem sintetik. Aneka spesis saling mengeksploitasi dan melengkapi aktiviti metabolit yang digunakan untuk pertumbuhan, survival dan pembiakkan alga. Kajian ini menggunakan eutrofikasi alga dari kolam ikan talapia, mengambil manfaat daripadanya dan mengenali potensi alga untuk bukan sahaja menjadi alternatif biofuel, malah ianya boleh menjadi makanan haiwan dan makanan tambahan manusia. Objektif kajian ini adalah; untuk mengetahui produktiviti dan kadar pertumbuhan spesifik; untuk memeriksa diversiti dan mengenal pasti spesis alga, untuk mengkuantifikasi komposisi biokimia dan ketoksikan alga campuran yang telah dikultur dan dituai sepanjang tiga jenis cuaca.

Produktiviti dan kadar pertumbuhan spesifik telah diuji menggunakan tiga jenis analisis iaitu; ketumpatan optik (680nm), berat kering biomas, dan klorofil *a*. Anggaran kepekatan spesis telah diukur menggunakan slaid pengiraan Nebauer dan alga campuran yang dikultur telah divisualkan menggunakan mikroskop bercahaya Olympus BH2 pada kadar pembesaran 400× dan 1000×. Kebanyakkan diatom dan dinoflagellate telah dikenal pasti pada tahap spesis dengan menggunakan buku rujukan taksonomi alga. Analisis komposisi biokimia yang telah dijalankan adalah; protein terlarut, kaedah Bradford; karbohidrat, kaedah kabohidrat terlarut; dan lipid, kaedah Bligh dan Dyer yang telah diubahsuai. Analisis kadar ketoksikan telah diuji dengan cara mendedahkan ekstrak alga kepada udang air garam (*Artemia nauplii*).

Satu kajian telah dijalankan terlebih dahulu, dimana campuran kepekatan alga dan air telah diuji. Berdasarkan hasil dari kajian awal, dua kepekatan telah menunjukkan hasil produktiviti dan kadar pertumbuhan alga tertinggi iaitu 50 % v/v dan 75 % v/v dan ianya telah dipilih untuk dikultur sepanjang kajian ini. Proses pengkulturan telah dibuat pada tiga jenis cuaca; hujan; dimana kultur mengalami hujan pada setiap hari sepanjang proses pengkulturan, cuaca campuran; dimana kultur hanya mengalami hujan untuk beberapa hari sepanjang proses pengkulturan, dan cuaca cerah; dimana kultur tidak mengalami hujan sepanjang proses pengkulturan. Kadar produktiviti tertinggi; 0.04 ± 0.00 g L^{-1} d^{-1} dan kadar pertumbuhan tertinggi; 0.21 ± 0.02 μ d^{-1} yang telah direkod adalah daripada kultur yang menggunakan kepekatan 75 % v/v semasa cuaca cerah. Sepanjang tempoh 30 hari dari proses pengkulturan (untuk tiga jenis cuaca); 4 jenis spesis telah dikenal pasti semasa cuaca hujan, 8 jenis spesis telah dikenal pasti semasa cuaca campuran dan 12 jenis spesis telah dikenal pasti semasa cuaca cerah. Beberapa spesis wujud semasa ketiga-tiga jenis cuasa, menjadikan jumlah keseluruhan 13 spesis yang telah dikenalpasti. Berdasarkan analisis biokimia, kandungan protein tertinggi (%); 46.88±0.23, kandungan karbohidrat (%); 31.36±0.21 dan kandungan lipid (%); 29.41±0.24 iaitu kesemuanya telah dikultur menggunakan kepekatan 75 % v/v semasa cuaca cerah. Ujian ketoksikan membuktikan alga campuran yang telah dikultur adalah sesuai untuk dimakan. Apabila *Artemia nauplii* didedahkan kepada ekstrak alga campuran 30 mg L^{-1}, nilai LD_{50} ialah 59.33 % dan ianya dianggap tidak toksik.

Apabila kultur mendapat sinaran cahaya matahari yang mencukupi, keadaan kultur yang optimum, dan digabungkan dengan keadaan sekeliling yang kondusif akan menghasilkan produktiviti mikroalgae campuran yang tinggi dengan potensi tidak terbatas. Walaubagaimanapun, pengkulturan mikroalga campuran dan faktor-faktor lain yang mempengaruhi proses ini boleh dikaji dengan lebih lanjut pada masa akan datang untuk mengetahui dengan lebih mendalam jenis aspek yang mempengaruhi kadar pertumbuhan alga jika kondusi persekitaran kultur diubah.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my supervisor Dr. Hishamuddin bin Omar for the continuous support throughout my Masters study, for his patience, motivation, and immerse knowledge. I appreciate his encouragements for enlightening me the first glance of research. His guidance helped me during the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Masters study. I would like to express my appreciation to my thesis committee; Prof. Dr. Ahmad bin Ismail not only for his insightful comments, but also for his eye opening questions which intrigued me to widen my research from various prospective.

My sincere gratitude also goes to Mr. Helmy Rozario, Mr. Kamal Khamis, and Mr Radin who has provided me an opportunity and access to the Plant Physiology Lab and Taman Pertanian Universiti research facility. Without their approval it would impossible to complete this research. I am also grateful for my fellow labmates for the stimulating discussions, for the sleepless nights working together before deadlines and for all the research experience we gained for the past two years. Last but not least, I would like to express paramount gratitude to my family; my parents and my brother for their emotional and spiritual support throughout the completion of my Masters research.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Hishamuddin Bin Omar, PhD
Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Ahmad bin Ismail, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :

Declaration by graduate student

I hereby confirm that:
- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: ______________________________ Date: ________________

Name and Matric No.: Tunku Ainna Binti Tunku Azmir Shah / GS39051

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature:	
Name of Chairman of Supervisory Committee:	Dr. Hishamuddin Bin Omar

Signature:	
Name of Member of Supervisory Committee:	Professor Dr. Ahmad bin Ismail

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

g	grams
mg	milligram
L	litre
mL	millilitre
°C	degree Celcius
µmol	micromol
m	meter
mm	millimeter
cm	centimeter
nm	nanometer
MPa	megapascal
s	second
min	minutes
d	days
v	volume
rpm	rotation per minute
sp.	species
%	percent
CO_2	carbon dioxide
LD_{50}	Lethal Dose $_{50}$
UV	ultraviolet
SE	standard error

SPSS	Statistical Package for Social for the Social Science
ANOVA	Analysis of Variance

CHAPTER 1

INTRODUCTION

The world at present is facing a serious climate problem. Greenhouses gases such as CO_2 and CH_4 released to the earth's atmosphere as the results of everyday living, will form an invisible blanket which traps heat thus causing gradual warming of the Earth. Apart from global warming, air pollution from industrial and vehicular emissions indirectly causes water pollution. Apart from that, domestic and industrial effluent, raw sewage, deforestation and other human activities also deteriorate natural and manmade water bodies. Environmental pollution created by human hosts other problems associated with environmental degradation which will lead to malnutrition due to soil depletion and insufficiency of fertile agriculture crop. These conditions could be worse if there are no control measures taken.

These predicaments could be reduced with the aid of microalgae cultivation. Combination of CO_2 fixation, biofuel production, and wastewater treatment may provide a very promising alternative to current CO_2 mitigation strategies. The age of cheap petroleum will end when crude oil reserve exhausted in near future. According to analysts, at the present rate of consumption, the fossil fuel reserves will be exhausted within just 50 years (Rodolfi et al., 2009). Microalgae have the potential to emerge as an unconventional sustainable resource because of their neutrality towards natural environment and flexible cultivation (Chen et al., 2015). Microalgae have the ability to convert sunlight, carbon dioxide, water and inorganic fertilizers into useful products such as proteins, lipids, carbohydrates, pigments, vitamins, and the oxygen released from algal cultivation could be used as biomitigation strategy in eliminating excessive amount of CO2 within earths' atmosphere. Their commercial potential has attracted the attention of biochemists, microbiologists, chemical engineers, and most recently, entrepreneurs.

The enrichment of water bodies will cause accelerated growth of algae which produce undesirable disturbance to the balance of organism present in the water and the quality of water is concerned. Eutrophication will boosts the growth of microalgae resulting in reduced water clarity and increased biological oxygen demand and the reduced dissolve oxygen will cause death to aquatic organisms. However, increased microalgae productivity due to eutrophication could have positive outcomes which are often overlooked. These microalgal blooms can be harvested and the biomass can be converted and utilized into human food, animal feed, source of fine chemicals (astaxanthin, beta carotene, and chlorophyll) and also biofuel. The irony is that despite of its potential, most scientist and common people look at microalgae bloom as environmental problem. However, by exploiting mixed microalgae culture derived from eutrophication may be an alternative solution for water contamination, which will then have the capacity for commercialization (Venkata Mohan et al., 2011).

On the opposite side of microalgae bloom as a form of pollution, mainstream research on microalgae as potential water bioremediator, CO_2 sequestration, biomass production and biofuel emphasizes more on laboratory approach. Most efforts are dedicated to find specific microalgae species and finding suitable biomass production protocol for biofuel, culture studies, and algae as carbon source in growth media (Mutanda et al., 2011). Although few species were found suitable for cultivation but scaling up to mass production level and contamination still a major issue. The crisis with pure culture is contamination that transpires because of open pond system that could not be controlled compared to photobioreactor systems. Cost of culturing monospecies in those systems is still prohibitively expensive. Due to this circumstance, limitations exist in many researches and predicaments arise where they are unable to produce in large scale and contaminations would occur in large batches of microalgae production.

The appeal of microalgae is that sunlight energy and carbon sources could be substituted for the expensive fermentable substrates required in industrial microbiology. Even when using controlled closed system fermenters (photobioreactor), contaminations could only take place in prolonged periods of fermentation. Positively, mixed microalgae could be cultivated in an open pond system at a very low cost due to the usage of naturally available sunlight and CO_2. It is also more practical, easily maintain and low capital input which offset its high productivity. Ecosystems with greater number of species are more stable and more resilient to change than monoculture systems made up of just one crop (Kazamia et al., 2012).

The leads of this study are; the low capital investment, low capital expenditure, the robust growth mixed microalgae culture in changing culture conditions and the algal diversity that it offers. Changing culture conditions is quite prominent due to geographical location of Malaysia which is 3°N approaching the equator line. Malaysia has no definite seasons like monsoon or temperate. However, Malaysia experience weather conditions changes such as sunny, rainy and mixed weather (sunny and rainy). Algae species within the mixed microalgae culture may experience diversity fluctuation due to these weather changes. As the results, there will be different dominant species occurring during different times throughout cultivation period. Advantages of commissioning microalgal cultures over agricultural systems are the very high yields of photosynthetic efficiencies and the usage of saline waters and marginal lands. The ability to manipulate the metabolism of microalgae through control of the environment may result in appealing outcome (Benemann and Tillett, 2009).

The objectives of this study are;

i. to measure the productivity and the Specific Growth Rate (SGR) of mixed microalgae cultured in outdoor open pond system based on optical density, dry weight and chlorophyll *a*.
ii. to examine the algal succession and species identification of mixed microalgae cultured in outdoor open pond system,
iii. to proximate the biochemical composition and the toxicity of the harvested mixed microalgae throughout three different weather conditions during the cultivation process.

Thus, by using mixed microalgae, understanding the ecosystem via observation and close monitoring, will promote microalgae growth and production in outdoor variable weather conditions.

REFERENCES

Abeliovich, A. and Weisman, D. (1978) Role of heterotrophic nutrition in growth of the alga *Scenedesmus obliquus* in high-rate oxidation ponds. *Applied Environmental Microbiology* 35: 32-37.

Abou-Shanab, R., Matter, I. A., Kim, S., Oh, Y., Choi, J. and Jeon, B. (2011) Characterization and identification of lipid producing microalgae species isolated from freshwater lake. *Biomass and Bioenergy* 35: 3079-3085.

Abou-Shanab, R.A.I., Jeon, B.H., Song, H., Kim, Y. and Hwang, J. (2010) Algae-Biofuel: Potential use as sustainable alternative green energy. *The Journal on Power and Energy Engineering* 1(1): 4–6.

Addy, K., and Green, L., (1996) Algae in aquatic ecosystems. *Natural resources facts* pp 96-104.

Adessi, A., Torzillo, G., Baccetti, E. and De Philippis, R. (2012) Sustained outdoor H_2 production with *Rhodopseudomonas palustris* cultures in a 50 L tubular photobioreactor. *International Journal of Hydrogen Energy* 10: 8840–8849.

Adhikary S.P. and Sahu, J.K. (2000) Survival strategies of Cyanobacteria occurring as crust in the rice field under drought conditions. *Indian Journal of Microbiology* 40: 53-56.

Al-Gabr, H.M., Zheng, T. and Yu, X. (2014) Efficiency of two chemical coagulants and three different filtration media on removal of *Aspergillus flavus* from surface water. *Journal of Environmental Science* 26: 274-280.

Aljuboori, A.H.R., Idris, A., Abdullah, N. and Mohamad, R. (2013) Production and characterization of a bioflocculant produced by *Aspergillus flavus. Bioresource Technology* 127:489-493.

Al-Shayji, Y.A., Puskas, K., Al-Daher, R. and Esen, I.I. (1994). Production and separation of algae in a high-rate ponds system. *Environment International* 20 (4): 541–550.

Anderson, R.A., and Preisig H.R. (2005) Historical Review of Algal Culturing Technique. In: Anderson RA, editor. *Algal culturing techniques* pp1-13. Elsevier Academic Press.

Aslan, S. and Kapdan, I.K. (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering* 28(1): 64–70.

Azov, Y. (1982) Effect of pH on inorganic carbon uptake in algal cultures. *Applied Environmental Microbiology* 43(6): 1300-1306.

Azov, Y. and Goldman, J.C. (1982) Free ammonia inhibition of algal photosynthesis in intensive cultures. *Applied Environmental Microbiology* 43: 735-739.

Azov, Y., Shelef, G. and Moraine, R. (1982) Carbon limitation of biomass production in high-rate oxidation ponds. *Biotechnology Bioengineering* 24: 579–594.

Backlund, P., A. Janetos, D. Schimel, J. Hatfield, K. Boote, P. Fay, L. Hahn, C. Izaurralde, B.A. Kimball, T. Mader, J. Morgan, D. Ort, W. Polley, A. Thomson, D. Wolfe, M. Ryan, S. Archer, R. Birdsey, C. Dahm, L. Heath, J. Hicke, D. Hollinger, T. Huxman, G. Okin, R. Oren, J. Randerson, W. Schlesinger, D. Lettenmaier, D. Major, L. Poff, S. Running, L. Hansen, D. Inouye, B.P. Kelly, L Meyerson, B. Peterson, and R. Shaw. (2008) *The Effects of Climate Change on Agriculture, Land Resources, Water Resources, and Biodiversity in the United States.* A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. U.S. Environmental Protection Agency, Washington, DC, USA.

Baek, S.H., Kim, D., Son, M., Yun, S.M. and Kim, Y.O. (2015) Seasonal distribution of phytoplankton assemblages and nutrient enriched bioassays as indicators of nutrient limitation of phytoplankton growth in Gwangyang Bay, Korea. *Estuary Coastal Shelf Sciences* pp11-14.

Bahadar, A. and Bilal Khan, M. (2013) Progress in energy from microalgae: A review. *Renewable and Sustainable Energy Review* 27: 128-148.

Bahl, J., Lau, M.C.Y., Smith, G.J.D., Vijaykrishna, D., Cary, S.C., Lacap, D.C., Lee, C.K., Papke, R.T., Warren-Rhodes, K.A., Wong, F.K.Y., McKay, C.P. and Pointing, S.B. (2011) Ancient origins determine global biogeography of hot and cold desert cyanobacteria. *Nature Communications* 2: 163.

Béchet, Q., Shilton, A. and Guieysse, B. (2013) Modeling the effects of light and temperature on algae growth: State of the art and critical assessment for productivity prediction during outdoor cultivation. *Biotechnology Advances* 31(8): 1648-1663.

Becker, E.W. (2008) Microalgae: Biotechnology and Microbiology. pp 112-116. Cambridge University Press.

Becker, W. (2004) Microalgae for aquaculture. The nutritional value of microalgae for aquaculture. Handbook of Microalgae Culture. pp 380-391. Blackwell, Oxford.

Behrens, P.W. (2005). Photobioreactor and fermentors: the light and the dark sides of the growing algae. In: Andersen, R.A. (Ed.), Algal Culturing Techniques. pp. 189-204. Elsevier Academic Press, New York, USA.

Bellinger, E.G. and Sigee, D.C. (2010) Freshwater Algae: Identification and Uses as Bioindicators. pp 1-40. John Wiley and Sons Ltd.

Benemann J.R. and Tillett D.M. (1987) Effects of fluctuating environments on the selection of high yielding microalgae. pp 7-10. Atlanta, USA.

Benemann, J. R. (2009) Microalgae Biofuels, A Brief Introduction. pp 34-50. Benemann Associates and MicroBio Eng. Walnut Creek, CA.

Bergstrom, C., McKeel, C. and Patel, S. (2007) Effects of pH on algal abundance: A model of Bay Harbor, Michigan. pp 12-15 University of Michigan.

Bertozzini, E., Galluzzi, I., Ricci, F., Penna, A., and Magnani, M. (2013) Neutral lipid content and biomass production in *Skeletonema marinoi* (Bacillariophyceae) culture in response to nitrate limitation. *Applied Biochemistry and Biotechnol*ogy 170: 1624-1634.

Bezawada, J., Hoang, N.V., More, T.T., Yan, S., Tyagi, N., Tyagi, R.D. and Surampalli, R.Y. (2013) Production of extracellular polymeric substances (EPS) by *Serratia* sp.1 using wastewater sludge as raw material and flocculation activity of the EPS produced. *Journal of Environmental Management* 128: 83-91.

Biller, P. and Ross, A.B. (2014) Pyrolysis GC-MS as a novel analysis technique to determine the biochemical composition of microalgae. *Algal Research* 6: 91-97.

Blankena, W., Cuaresma, M., Wijffels, R.H. and Janssen, M. (2013). Cultivation of microalgae on artificial light comes at a cost. *Algal Research* 2: 333–340.

Bligh, E. G and Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. *Journal of Biochemistry and Physiology* 37: 911-917.

Bono Jr., M.S., Ahner. and Kirby, B.A. (2013) Detection of algal lipid accumulation due to nitrogen limitation via dielectric spectroscopy of *Chlamydomonas reinhardtii* suspensions in a coaxial transmission line sample cell. *Bioresource Technology* 143: 623–631

Borowitzka, L.J. (1991). Development of Western biotechnology algal beta-carotene plant. *Bioresource Technology* 38: 251–252.

Borowitzka, L.J., and Borowitzka, M.A. (1989) ß-carotene (provitamin a) production with algae. In: Vandamme EJ (ed) Biotechnology of vitamins, pigments and growth factors. *Applied Science* 29: 15–26.

Borowitzka, M. A. (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology* 70: 313-321.

Borowitzka, M.A. (1998). Algae as food. In Microbiology of fermented foods, 2nd ed, ed. B.J.B. Wood. pp 585–602. Blackie Academic and Professional.

Borowitzka, M.A. (2012) Australian Biotechnology and Environment Application for Microalgae: *Microalgae History* pp 47-82.

Borowitzka, M.A. and Moheimani, N.R. (2010) Sustainable biofuels from algae.Mitigation and Adaptation Strategies for Global Change. pp 1–13. Retrieved from: 10.1007/s11027-010-9271-9.

Bouaicha, N., Rivasseau, C., Hennion, M.C. and Sandra, P. (1996) Detection of cyanobacterial toxins (microcystins) in cell extracts by micellar electrokinetic chromatography. *Journal of Chromatography* 685: 53-57.

Boussiba, S., Sandbank, E., Shelef, G., Cohen, Z., Vonshak, A., Ben Amotz, A., Arad, S. and Richmond, A. (1988) Outdoor cultivation of the marine microalga *Isochrysis galbana* in open reactors. *Aquaculture* 72: 247–253.

Boyd, C.E. (1970) Amino acid, protein and coloric content of vascular aquatic macrophytes. *Ecology* 51: 902-906

Bradford, M.M. (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizinf principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Bratskaya, S., Schwarz, S., Liebert, T. and Heinze, T. (2005) Starch derivatives of high degree of functionalization: 10. Flocculation of kaolin dispersions. Colloids Surf. *Physicology Engineering Aspect* 254: 75-80.

Brennan, L. and Owende, P. (2010) Biofuels from microalgae - a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Review* 14: 557-577.

Brinkman, R. and Sombroek, W.G. (1995) Global climate change and agricultural production: The effects of global change on soil conditions in relation to plant growth and food production. pp 23-42.

Brock, T.D. (1973) Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. *Science* 179: 480-483.

Brown, M.R., Jeffrey, S.W. and Garland, C.D. (1989) Nutritional aspects of microalgae used in mariculture: a literature review. C.S.I.R.O. *Marine Laboratories Report* 205: 1–44.

Brown, M.R., Jeffrey, S.W., Volkman, J.K. and Dunstan.G.A. (1997) Nutritional properties of microalgae for mariculture. *Aquaculture* 151: 315–331.

Brune, D.E., Schwartz, G., Eversole, A.G., Collier, J.A. and Schwedler, T.E. (2003) Intensification of pond aquaculture and high rate photosynthetic systems. *Aquaculture Engineering* 28: 65-86.

Campbell, P.K., Beer, T. and Batten, D. (2011) Life cycle assessment of biodiesel production from microalgae in ponds. *Bioresource Technology* 102(1): 50–56.

Cea-Barcia, G., Buitrón, G., Moreno, G. and Kumar, G. (2014) A cost effective stratergy for the bio-prospecting of mixed microalgae with high carbohydrate content: Diversity fluctualtions in different growth media. *Bioresource Technology* 163: 370-373.

Celekli, A. and Dönmez, G. (2006) Effect of pH, light intensity, salt and nitro-gen concentration on growth and β-carotenes accumulation by a new isolate *Dunaliell*a sp. *World Journal of Microbiology and Biotechnology* 22: 183-189.

Celekli, A., Yavuzatmaca, M. and Bozkurt, H. (2009) Modeling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes. *Bioresource Technology* 100: 3625-3629.

Chakraborty, R.D., Chakraborty, K. and Radhakrishnan, E.V. (2007) Variation in fatty acids composition of *Artemia salina* nauplii enriched with microalgae and baker's yeast for use in larviculture. *Journal of Agriculture and Food Chemistry* 55: 4043–4051.

Challinor, A.J., Simelton, E.S., Fraser, E.D.C., Hemming, D. and Collins, E. (2010) Increased crop failure due to climate change: assessing adaptation options using models and socio-economic data for wheat in China. *Environment Research Letters* 5(3): 3058-3060.

Chapman, H. and Leusch, F. (2011) SEQ urban water security research alliance: bioassay and risk communication. pp 34-37.

Cheirsilp, B. and Suwannarat, W. (2011) Mixed culture of oleaginous yeast *Rhodotorula glutinis* and microalga *Chlorella vulgaris* for lipid production from industrial wastes and its use as biodiesel feedstock. *New Biotechnology* 110: 510–516.

Chen, F. (1996). High cell density culture of microalgae in heterotrophic growth. *Trends Biotechnology* 14: 412-426.

Chen, G., Zhao, L. and Qi, Y. (2015) Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: A critical review. *Applied Energy* 137: 282-291.

Chen, M., Tang, H., Ma, H., Holland, T.C., Ng, K.Y.S. and Salley, S.O. (2011) Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. *Bioresource Technology* 102: 1649-1655.

Chen, W.T., Zhang, Y., Zhang, J., Yu, G., Schidenman, L.C., Zhang, P. and Minarick, M. (2014) Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil. *Bioresource Technology* 152: 130-139.

Chia, A.M., Bako, S.P., Alonge, S., and Adamu, A.K. (2011) Green algal interactions with physicochemical parameters of some manmade ponds in Zaria, northern Nigeria. *Revista Brasil Botanica* 34(3): 285-295.

Chioccioli, M., Hankamer, B. and Ross, I.L. (2014) Flow Cytometry Pulse Width Data Enables Rapid and Sensitive Estimation of Biomass Dry Weight in the Microalgae *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. pp 176-188.

Chisti, Y. (2008) Biodiesel from microalgae beats bioethanol. *Trends Biotechnology* 26: 126–131.

Christi, Y. (2007) Biodiesel from microalgae. *Biotechnology Advances* 25: 294-306.

Clarens, A.F., Resurreccion, E., White, M. and Colosi, A. (2010) Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental Science and Technology* 44: 1813–1819.

Connell, J.H. and Slatyer, R.O. (1977) Mechanism of sucession in natural communities and their rolein community stability and organization. *American Naturalist* 111: 1119-1144.

Converti, A. Casazza, A.A., Ortiz, E.Y., Perego, P. and Del Borghi, M. (2009) Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering Processing: Process Intensification* 48: 1146-1151.

Cooper, A. (1988) The system and Operation of the system. In: The ABC of NFT. Nutrient Film Technique, pp 3-123. Grower Books. London, England.

Cornet, J.F. (2010) Calculation of optimal design and ideal productivities of volumetrically lightened photobioreactors using the constructal approach. *Chemical Engineering Sciences* 65(2): 985–998.

Danesi, E.D.G., Rangel-Yagui, C.O., Carvalho, J.C.M. and Sato, S. (2004) Effect of reducing the light intensity on the growth and production of chlorophyll by *Spirulina platensis*. *Biomass and Bioenergy* 26: 329-335.

Danielo, O. (2005) An algae-based fuel. *Biofuture* pp 225-226

Dhont, J. and Van Stappen, G. (2003) Live feeds in marine aquaculture. Blackwell Science Ltd. pp 65–121.

Dormer, C. (2012) Natural Environment Research Council: A case of mixed microalgae. pp 152-166.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28 (3): 350–356.

El-Sharraf, W.M. and El-Shaarawy, G. (1994) Chemical composition of some marine algae from the Mediterranean Sea of Alexandria, Egypt. *The Bulletin of Marine Ecosystem* 3(24): 523-534.

El-Skeekh, M., Abomohra, A.E.F. and Hanelt, D. (2013) Optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as promising microalga for biodiesel production. *World Journal of Microbiology and Biotechnology* 29: 915-922.

Enright, C.T., Newkirk, G.F., Craigie, J.S. and Casteell, J.D. (1986) Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. *Journal of Experimental Marine Biology and Ecology* 96: 1–13.

Environment Protection Agency (2008) Coastal Zones and Sea Level Rise. pp 22-31.

Epifanio, C.E. (1979) Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea Íirginica* (Gmelin) and the hard clam *Mercenaria mercenaria*. *Aquaculture* 18: 187–192.

Epifanio, C.E. (1983) Phytoplankton and yeast as food for juvenile bivalves: a review of research at the University of Delware. In: Langdon, C.L., and Conkin, D.E. (Eds)., Proceedings of 2nd International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition, Louisiana State University, Baton Rouge. pp 292–304. Los Angeles, USA.

Fagiri, Y.M.A, Salleh, A. and El-Nagerabi, S.A.F. (2013) Influence of chemical and environmental factors on the growth performance of *Spirulina platensis* strain SZ100. *Journal of Algal Biomass Utilization* 4 (2): 7-15.

Feng, P., Deng, Z., Fan, L. and Hu, Z. (2012) Lipid accumulation and growth characteristics of *Chlorella zofingiensis* under different nitrate and phosphate concentration. *Bioscience Bioengineering* 114:405-410

Fidalgo, J.P., Cid, A., Lopez-Munoz, I., Abalde, A. and Herrero, C. (1994) Growth and biochemical profile of juvenile mussels (*Mytilus galloproÍincialis)* fed on different algal diets. *Journal of Shellfish Research* 1: 67–75.

Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White. (2014) Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, pp 1009-1132. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Fisher, T., Berner, T., Iluz, D., and Dubinsky, Z. (1998) The kinetics of the photoacclimation response of Nannochloropsis sp. (Eustigmatophyceae): A study of changes in ultrastructure and psu density. *Journal of Phycology* 34: 818–824.

Florentino de Souza Silva, A. P., Costa, M.C., Lopes, A.C., Neto, E.F.A., Leitão, R.C., Mota, C.R., and Bezerra dos Santos, A. (2014) Comparison of pretreatment methods for total lipids extraction from mixed microalgae. *Renewable Energy* 63: 762-766

Gallagher, S.J. and Sommerville I.D. (2003) Lower carboniferous platform development and cyclicity in southern Ireland: foraminiferal biofacies and lithofacies evidence. *Rivista Italiana di Paleontologist Stratigrafa* 109: 152-165.

Gatenby, C.M., Orcutt, D.M., Kreeger, D.A., Parker, B.C., Jones, V.A. and Neves, R.J. (2003) Biochemical composition of three algal species proposed as food for captive freshwater mussels. *Journal of Applied Phycology* 15: 1–11.

Goldman, J.C. and Shapiro, M.R. (1973) Carbon dioxide and pH: effect on species succession of algae. *Limnology and Oceanography* 182: 306-307.

Goldman, J.C., Porcella, D.B., Middlebrooks, J.E., Daniel, F. and Toerien. (1971) The effect of carbon on algal growth- its relationship to eutrophication. *Utah Water Research Lab* 180: 228-254.

González-Fernández, C. and Ballesteros, M. (2012) Linking microalgae and cyanbacteria culture conditions and key enzymes for carbohydrate accumulation. *Biotechnology Advances* 30: 1655-1661.

Gopal, K. (2007) Enumeration of blue green algae (cyanobacteria) in water. *Water and Wastewater* pp 146-147.

Gouveia, L. (2011) Microalgae as a Feedstock for Biofuels, Springer Briefs in Microbiology. pp 44-73.

Graham, J.L., Loftin, K.A. and Kamman, N. (2009) Monitoring Recreational Freshwaters. Lakeline. pp 18-24.

Granum, E., Kirkvold, S. and Myklestad, S.M. (2002) Cellular and extracellular production of carbohydrates and amino acids by the marine diatom Skeletonema costatum: diel variations and effects of N depletion. *Marine Ecology Progress Series* 242: 83–94.

Grobbelaar, J.U. (2000) Physiological and technological considerations for optimizing mass algal cultures. *Jounal of Applied Phycology* 12: 201–206.

Guterman, H., Vonshak, A. and Ben-Yakkov, S. (1990) A macromodel for outdoor algal mass production. *Biotechnology Bioengineering* 35: 809–819.

Hampel, N., Petrick, I. and Behrendt, F. (2012) Biomass productivity of fatty acids and amino acids of microalgae strains as key characteristics of sustainability for biodiesel production. *Jounal of Applied Phycology* 24(6): 1407-1418.

Hanahan, D. (1985) Techniques for Transformation of E. coli DNA cloning. *Journal of DNA Research* 1: 109-135.

Hansmann, E. (1973) Pigment analysis. In Handbook of Phycological Methods, pp 359-368. Cambridge University Press, London.

Harun, I., Yahya, L., Chik, M.N., Kadir, N.N.A. and Mohd, M.A. (2014). Effects of Natural Light Dilution on Microalgae Growth. *International Journal of Chemical Engineering and Applications* 5(2): 112-116.

Hawkins, P.R., Holliday, J., Katruria, A. and Bowling, L. (2005) Change in cyanobacterial biovolume due to preservation by lugol's iodine. *Harmful Algae* 4:1033-1043.

Held, P. (2011) Monitoring of Algal Growth Using their Intrinsic Properties, Use of a Multi-Mode Monochromator-based Microplate Reader for Biofuel Research. *Biofuel Research* pp 1-5.

Hemaiswarya, S., Raja, R., Ravi Kumar, R., Ganesan, R. V. and Anbazhagan, C. (2011). Microalgae: a sustainable feed source for aquaculture World. *Journal of Microbiology and Biotechnology* 27: 1737–1746.

Herrero, C., Cid, A., Fabregas, J. and Abalde, J. (1991) Yields in biomass and chemical constituents of four comercially important marine microalgae with different culture media. *Aquacultural Engineering* 10: 99–110.

Hidu, H. and Ukeles, R. (1962) Dried unicellular algae as food for larvae of the hard shell clam, *Mercenaria mercenaria*. *Shellfisheries Assessment* 53: 85-101 .

Hu, H. and Zhou, Q. (2010) Regulation of inorganic carbon acquisition by nitrogen and phosphorus levels in the *Nannochloropsis* sp. *World Journal of Microbiology and Biotechnology* 26: 957–961.

Huertas, I.E., Rouco, M., Lo´pez-Rodas, V. and Costas, E. (2011) Warming will affect phytoplankton differently: evidence through a mechanistic approach. *Proceeding for Biology Sciences and the Royal Society* 278: 3534–3543.

Huisman, J. and Sommeijer, B. (2002) Maximal sustainable sinking velocity of phytoplankton. *Marine Ecology Progress Series* 244: 39-48.

Javanmardian, M. and Palsson, B.O. (1991) High-density photoautotrophic algal cultures: design, construction, and operation of a novel photobioreactor system. *Biotechnology Bioengineering* 38: 1182-1189.

Jefferey S.W. and Humphrey G.F. (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae, and natural phytoplankton. *Biochemical Physiology* 167: 191-194.

Jensen, S. and Knusten, G. (1993) Influence of light and temperature on photoinhibition of photosynthesis in *Spirulina platensis*. *Journal of Phycology* 5: 495-504.

Jeon, Y.C., Cho, C.W. and Yun, Y.S. (2005) Measurement of microalgal photosynthetic activity depending on light intensity and quality. *Biochemical Engineering Journal* 27(2): 127-131.

Jiméneza, C., Belén, R., Cossíob, F. and Niella, X. (2003) Relationship between physicochemical variables and productivity in open ponds for the production of Spirulina: a predictive model of algal yield. *Aquaculture* 221: 331-345.

Johnson, M.K., Johnson, E.J., MacElroy, R.D., Speer, H.L. and Bruff, B.S. (1968) Effects of salts on the halophilic alga *Dunaliella viridis*. *Journal of Bacteriology* 95: 1461-1468.

Kaplan, D., Richmond, A.E., Dubinsky, Z. and Aaronson, S. (1986) Algal nutrition. In: Richmond, A. (Ed.), Handbook for Microalgal Mass Culture. CRC Press, Boca Raton. pp 147-198.

Karl, T.R., Melillo, J.M. and Peterson, T.C. (2009) *Global Climate Change Impacts in the United States* . United States Global Change Research Program. Cambridge University Press, New York, NY, USA.

Kazamia, E., Aldridge, D.C. and Smith, A.G. (2012) Synthetic ecology: A way forward for sustainable algal biofuel production. *Journal of Biotechnology* 162: 163-169.

Khan, S., Rashmi, A., Hussain, M.Z., Prasad, S. and Banerjee, U.C. (2009) Prospects of biodiesel production from microalgae in India. *Renewable and Sustainable Energy Review* 13: 2361-2372.

Kim, J.H., Abu Affan, M., Jang, J., Kang, M., Ko, A., Jeon, S., Oh, C., Heo, S., Lee, Y. Ju, S. and Kang, D. (2015) Morphological, molecular and biochemical characterization of astaxanthin-producing green microalga *Haematococcus* sp. KORDI03 (*Haematoccaceae*, Chlorophyta) isolated from Korea. *Journal of Microbiology and Biotechnology* 25(2):238-246.

Koch, A.L. (1994) Growth Measurement In: Methods for General and Molecular Bacteriology. *American Society for Microbiology* pp 248-227.

Kunlasak, K., Chitmanat, C., Whangchai1, N., Promya, J. and Lebel, L. (2013) Relationships of Dissolved Oxygen with Chlorophyll-a and Phytoplankton Composition in Tilapia Ponds. *International Journal of Geosciences* 4: 46-53.

Lacerda, S.R., Koening, M.L., Neumann-Leitao, S. and Flores-Montes, M.J. (2004) Phytoplankton nyctemeral variation at a tropical river estuary. *Brazilian Journal of Biology* 64:81-94.

Laing, I. and Verdugo, G. (1991) Nutritional value of spray dried *Tetraselmis suecica* for juvenile bivalves. *Aquaculture* 92: 207–218.

Lastarria-Cornheil, S. and Manji, A. (2010) The state of food and agriculture: Land tenure, land policy, and gender in rural areas. *Food and Agriculture Organization of the United Nations* pp 63-81.

Leavitt, P.R., Findlay, D.L., Hall, R.I. and Smol J.P. (1999) Algal responses to dissolved organic carbon loss and pH decline during whole-lake acidification: Evidence from paleolimnology. *Limnology Oceanography* 44(32): 757–773.

Lee, K. and Lee, C.G. (2001) Effect of light/dark cycles on wastewater treatment by microalgae. *Biotechnology and Bioprocess Engineering* 6: 194-199.

Lee, K. and Lee, C.G. (2002) Nitrogen removal from wastewaters by microalgae without consuming organic carbon sources. *Journal of Microbiology and Biotechnology* 12: 979-985.

Lee, Y.K. (1986) Enclosed bioreactors for the mass cultivation of photosynthetic microorganisms: the future trend. *Trends Biotechnology* 4: 186–189.

Lee, Y.K. (2004) Algal nutrition. Heterotrophic carbon nutrition. In: Richmond, A. (Ed.), Handbook of Microalgal Culture. *Biotechnology and Applied Phycology* 232: 166-178.

Legendre, L., Rochet, M. and Demers, S. (1986) Sea-ice microalgae to test the hypothesis of photosynthetic adaptation to high frequency light fluctuations. *Journal of Experimental Marine Biology and Ecology* 97: 321-326.

Leon-Banares, R., Gonzalez-Ballester, D., Galvan, A. and Fernandez, E. (2004) Transgenic microalgae as green cell-factories. *Trends Biotechnology* 22(1): 45–52.

Levasseur, M., P.A. Thompson, and P.J. Harrison. (1993) Physiological acclimation of marine phytoplankton to different nitrogen sources. *Journal of Phycology* 29: 87-595.

Li, X., Xu, H., Chen, Z.S. and Chen, G. (2011) Biosynthesis of nanoparticlpes by microorganism and their applications. *Journal of Nanometer* pp 1-6.

Li, Y., Naghdi, F.G., Garg, S., Adarme-Vega, T.C., Thurecht, K.J., Ghafor, W.A., Tannock, S. and Schenk, P.M. (2014) A comparative study: the impact of different lipid extraction methods on current microalgal lipid research. *Microbiology and Cellular Facts* 18: 13-26.

Liang, S., Xueming, L., Chen, F. and Chen, Z. (2004) Current microalgal health food RandD activities in China. *Hydrobiologia* 512: 45–48.

Long, R.D. and Abdelkader, E. (2011) Mixed-polarity azeotropic solvents for efficient Extraction of Lipids from *Nannochloropsis* microalgae. *American Journal of Biochemistry and Biotechnology* 7(2): 70-73.

Lopes, E.J., Scoparo, C.H.G., Lacerda, L.M.C.F and France, T.T. (2008) Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. *Chemical Engineering Process Intensif* 54: 1-5.

Lopez-Elias, J. and Voltolina, D. (1993) Cultivos semicontinuos de cuatro especies de microalgas con un medio no convencional. *Ciencias Marinas* 19 (2): 169–180.

Love R.C., Loder, T.C. and Keafer, B.A. (2005) Nutrient conditions during *Alexandrium fundyense* blooms in the western Gulf of Maine, USA. *Deep-Sea Research II.* 52:2450–2466.

Lubzens, E., Gibson, O., Zmora, O. and Sukenik, A. (1995) Potential advantages of frozen algae (*Nannochloropsis sp.*) for rotifer (*Brachionus plicatilis*) culture. *Aquaculture* 133: 295–309.

Lum, K.K., Kim, J. and Lei, X.G. (2013) Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *Journal of Animal Science and Biotechnology* 4: 53-55.

Lundquist, T.J. (2010) A Realistic Technology and Engineering Assessment of Algae Biofuel Production, University of California, Berkley: Energy Bioscience Institute. pp 217-224.

Mahapatra, D.M., Chanakya, H.N. and Ramachandra, T.V. (2013) *Euglena* sp. as a suitable source of lipids for potential use as biofuel and sustainable wastewater treatment. *Journal of Applied Phycology* 156: 187-201.

Makareviciene, V., Skorupskaite, V. and Andruleviciute, V. (2013) Biodiesel fuel from microalgae- promising alternative fuel for future: A review. *Review Environmental Sciences and Biotechnology* 12: 119-130.

Markou, G.G., Chatzipavlidis, I. and Georgakakis, D. (2012) Cultivation of *Arthrospira* (*Spirulina) platensis* in olive-oil mill wastewater treated with sodium hypochlorite. *Bioresource Technology* 112: 234-241.

Masson, M. (1977) Observations on the feeding of larvae of *Mytilus galloprovincialis* with inert foods. *Marine Biology* 40: 157-164.

McGinn, P.J., Dickinson, K.E., Bhatti, S., Frigon J.C., Guiot, S.R., and O'Leary, S.J.B. (2011) Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. *Photosynthesis Research* 109: 231–47.

Metzger, P. and Largeau, C. (2005) *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Applied Microbiology and Biotechnology* 66 (5): 486-496.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nicholas, D.E. and McLaughlin, J.L. (1982) Brine shrimp: A convenient general bioassay for active plant constituents. *Journal of Medicinal Plant Research* 45: 31-34.

Miao, X. and Wu, Q. (2004) High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. *Journal of Biotechnology* 110: 85–93.

Milledge, J.J. and Heaven, S. (2012) A review of the harvesting micro-algae for biofuel production. *Review Environmental Science and Biotechnology* 12: 165-178.

Mock, T. and Kroon, B.M.A. (2002) Photosynthetic energy conversion under extreme conditions–I: important role of lipids as structural modulators and energy sink under N-limited growth in Antaric sea ice diatoms. *Phytochemistry* 61: 41–51

Mohan, S.V., Devi, M.P., Mohanakrishna, G., Amarnath, N., Babu, M.L. and Sarma, P.N. (2011) Potential of mixed microalgae to harness biodiesel from ecological water-bodies with simultaneous treatment. *Bioresource Technology* 102: 1109–1117.

Moheimani, N.R. (2013) Inorganic carbon and pH effect on growth and lipid productivity of *Tetraselmis suecica* and *Chlorella* sp. (Chlorophyta) grown outdoors in bag photobioreactors, *Journal of Applied Phycology* 19: 1–12.

Moheimani, N.R. and Borowitzka, M.A. (2007) Limits to productivity of the alga *Pleurochrysis carterae* (Haptophyta) grown in outdoor raceway ponds. *Biotechnology Bioengineering* 96: 27–36.

Müller,P., Xiao, P. and Niyogi, K.K. (2001) Non-photochemical quenching: A response to excess light energy. *American Society of Plant Physiologist* 125:4: 1558-1566.

Muller-Feuga, A. (2000) The role of microalgae in aquaculture: situation and trends. *Journal of Applied Phycology* 12: 527–534.

Muregi, F.W., Chhabra, S.C., Njagi, E.N.M., Langat Thoruwa, C.C., Njue, W.M., Orago, A.S.S., Omar, S.A. and Ndiege, I.O. (2004) Anti-plasmodial activity of some Kenyan medicinal plant extracts singly and in combination with chloroquinee. *Phytotherapy Research* 18: 379–384.

Mustafa, G.M. and Nakagawa, H. (1995) A review: Dietary benefits of algae as an additive in fish feed. Israeli. *Journal of Aquaculture-Bamidgeh* 47: 155–162.

Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A. and Fux, B (2011) Bioprospecting for hyper lipid producing microalgal strains for sustainable biofuel production. *Bioresource Technology* 102: 57-70.

Muthu, S.S. (2016) Carbon footprint handbook. CRC Press. pp16-17.

Myers, J.A., Curtis, B.S. and Curtis, W.R. (2013) Improving accuracy of cell and chromophore concentration measurements using optical density. *Biophysics* 76: 6-4

Napolitano, G.E., Ackman, R.G. and Ratnayake, W.M.N. (1990) Fatty acid composition of three cultured algal species (*Isochrysis galbana*, *Chaetoceros gracilis* and *Chaetoceros calcitrans*) used as food for bivalve larvae. *Journal of World Aquaculture Society* 21: 122–130.

Naqqiuddin, M.A., Nor, N.M., Omar, H. and Ismail, A. (2014) Development of simple floating photobioreactor design for mass culture of *Arthrospira platensis* in outdoor conditions: Effects of simple mixing variation. *Journal of Algal Biomass Utilization* 5(3): 46-58.

Nascimento, I.A., Cabanelas, I.T.D., Nunes dos Santos, J., Nascimento, M.A., Sousa, L. and Sansone, G. (2015) Biodiesel yields and fuel quality as criteria for algal-feedstock selection: Effects of CO_2 – supplementation and nutrient levels in cultures. *Algal Research* 8: 53-60.

National Science Foundation (2012) Microalgae history. Retrieved 21 June 2016 from http://hulab.ucf.edu/microalgae/introduction.

Nguta, J.M. and Mbaria, J.M. (2013) Brine shrimp toxicity and antimalarial activityof some plants traditionally used in treatment of malaria in Msambweni district of Kenya. *Journal of Ethnopharmacology* 148: 988-992.

O'Connor, W.A., Nell, J.A. and Diemer, J.A. (1992) The evaluation of twelve algal species as food for juvenile Sydney rock oysters *Sacccostrea commercialis* (Iredale and Roughley) larvae. *Aquaculture* 108: 277–283.

O'Rilley, A.M. and Scott, J.A. (1995) Define coimmobilization of mixed microalgae cultures. *Enzyme Microbiology Technology* 17: 636-646.

Oron, G., Shelef, G. and Levi, A. (1981) Environmental phenotypic variation of *Scenedesmus dimorphus* in high rate algae ponds and its relationship to wastewater treatment and biomass production. *Biotechnology Bioengineering* 23: 2185-2198.

Oswald, W.J. (1992) Micro-algae and waste-water treatment. In: Borowitzka, M.A., Borowitzka, L.J. (Eds.), *Microalgal Biotechnology* pp 305-328. Cambridge University Press, Cambridge, UK.

Oswald, W.J. and Golueke, C.G. (1960) Biological transformation of solar energy. *Sewage Industrial Wastes* 31: 1125–1142.

Oswald, W.J. and Gotaas, H.B. (1957) Photosynthesis in sewage treatment. *American Society Civil Engineering* 1957: 73–105.

Packer, M. (2009) Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. *Energy Policy* 37(9): 3428–37.

Pahl, S.L., Andrew K.L., Kalaitzidis, T., Ashman, P.J., Sathe, S. and Lewis, D.M. (2013) Harvesting, thickening and dewatering microalgae biomass. *Algae for Biofuels and Energy* pp165-185.

Parmar, A., Singh, N.K., Pandey, A. and Gnansounou, E. (2011). Cyanobacteria and microalgae: A positive prospect for biofuels. *Bioresource Technology* 102: 10163–10172.

Pedro, C. and Fernández-Díaz, J.C. (2001) Pilot evaluation of freeze-dried microalgae in the mass rearing of gilthead seabream (*Sparus aurata*) larvae. *Aquaculture* 193: 257–269.

Percival, E.G.V. and McDowell, R.H. (1967) Chemistry and Enzymology of Marine Algal Polysaccharides, pp 41-55. Academic Press. London, UK.

Perez-Garcia, O., Escalante, F.M.E., de-Bashan, L.E. and Bashan, Y. (2011) Heterotrophic cultures of microalgae: Metabolism and potential products. *Water Research* 45: 11–36.

Phatarpekar, P.V., Sreepada, R.A., Pednekar, C. and Achuthankutty, C.T. (2000) A comparative study on growth performance and biochemical composition of mixed culture of *Isochrysis galbana* and *Chaetoceros calcitrans* with Monocultures. *Aquaculture* 181: 141–155.

Ponis, E., Robert, R. and Parisi, G. (2003) Nutritional value of fresh and concentrated algal diets for larval and juvenile Pacific oysters (*Crassostrea gigas*). *Aquaculture* 221: 491–505.

Prathima Devi, M., Venkata Subhash, G. and Venkata Mohan, S. (2011) Heterotrophic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases: Effect of nutrient supplementation. *Renewable Energy* 43: 276-283.

Pratoomyot, J., Srivilas, P. and Noiraksar T. (2005) Fatty acids composition of 10 microalgal species. *Journal of Science and Technology* 27 (6):1179–1187.

Prochazkova, G., Kastanek, P. and Branyik, T. (2015) Harvesting freshwater *Chlorella vulgaris* with flocculant derives from spent brewer's yeast. *Bioresource Technology* 177: 28-33.

Proctor, V.W. (1957) Some controlling factors in the distribution of *Haematococcus pluvialis*. *Ecology* 38(3): 457-462.

Quinn, J., Winter, L. and Bradley, T. (2011) Microalgae bulk growth model with application to industrial scale system. *Bioresource Teachnology* 102: 5083-5092.

Radmer, R.J. and Parker, B.C. (1994) Commercial applications of algae: opportunities and constraints. *Journal Applied Phycology* 6: 93-98.

Rai, H. (1973) Methods involving the determination of photosynthetic pigments using spectrophotometry. *Limnnology* 18: 1864-1875.

Rao, A.R., Ravishankar, G.A. and Sarada, R. (2012) Cultivation of green alga *Botryococcus braunii* in raceway, circular ponds under outdoor conditions and its growth, hydrocarbon production. *Bioresource Technology* 123: 528–533.

Ras, M., Steyer, J.P. and Bernard, O. (2013) Temperature effect on microalgae: a crucial factor for outdoor production. *Review Environmental Science and Biotechnology* 12: 153–164.

Rawat, I., Ranjith Kumar, R., Mutanda, T., and Bux, F. (2011) Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy* 88: 3411–3424.

Raymont, J.E.G. (1963) Plankton and Productivity in Oceans. Oxford Press. pp 30-35.

Renaud, S.M., Parry, D.L. and Thinh, L.V. (1999) The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture* 170: 147-159.

Reynolds, C.S. (1984) The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge pp 20-23.

Richmond A. (2004) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd. pp 74-77.

Richmond, A. (1986) Handbook of Microalgal Mass Culture. CRC, Boca Raton. pp 528-551.

Richmond, A. (1992) Open systems for the mass production of photoautotrophic microalge outdoors: physiological principles. *Journal Applied Phycology* 4: 281–286.

Robarts, R. D. and Zohary, T. (1987) Temperature Effects on Photosynthetic Capacity, Respiration and Growth Rates of Bloom-Forming Cyanobacteria. *New Zealand Journal of Marine and Freshwater Research* 2(11): 65-82.

Rodolfi, L. Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M.R. (2009) Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in low cost photobioreactor. *Biotechnology Bioengineering* 102 (1): 100-112.

Romberger, H.P. and Epifanio, C.E. (1981) Comparative effects of diets consisting of one or two algal species upon assimilation efficiencies on growth of juvenile oysters, *Crassostrea Íirginica* (Gmelin). *Aquaculture* 25: 77–87.

Ryther, J.H. and Goldman, J.C. (1975) Microbes as food in mariculture. *American Reveiw Microbiology* 29: 429-443.

Sahoo, D. and Seckbach J. (2015) The algae world. Springer Science. pp 359-362.

Samuelsson, G., Lönneborg, A., Rosenqvist, E., Gustafsson, P. and Öquist, G. (1985) Photoinhibition and reactivation of photosynthesis in the cyanobacterim *Anacystis nidulans. Plant Physiology* 79: 992-995.

Sayre, R. (2010) Microalgae: The Potential for Carbon Capture. *Bioscience* 9: 60-61.

Schulz, T. (2006) The economics of microalgae production and processing into biofuel. Department of Agriculture and Food, Government of Western Australia. pp 92-105.

Shapiro, J. (1973) Blue-green algae: why they become dominant. *Science* 179: 382-384.

Sheehan, J., Dunahay, T., Benemann, J. and Roessler, P. (1998) A look back at the U.S. Department of Energy's aquatic species program: biodiesel from algae. *National Renewable Energy Laboratory* 5: 179-181.

Shiraiwa, Y., A. Goyal, A. and Tolbert, N.E. (1993) Alkalization of the medium by unicellular green algae during uptake of dissolved inorganic carbon. *Plant Cell Physiology* 34: 649–657.

Shoaf, W., Thomas, A. and Lium, B.W. (1976) Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. *Limnology Oceanography* 6: 112-145.

Shor, S. (2011) Children at risk for poor nutrition: expending the approach of future professionals in educational institutions. *Child Abuse and Neglect* 35: 606-612.

Simpson, A. (1991) Volunteer lake monitoring: A method manual. pp 91-102.

Singh, P.K. (1974) Effect of pH on Growth and Nitrogen Fixation in Aphanothece (Cyanophyta). *Oikos* 25 (1):114-116.

Singh, S.P and Singh, P. (2004) Effect of CO_2 concentration on algal growth: A review. *Renewable and Sustainable Energy Reviews* 38: 172–179.

Smith, V.H., Sturm, B.S.M., DeNoyelles, F.J. and Billings, S.A. (2009) The ecology of algal biodiesel production. *Trends in Ecology and Evolution* 25(6): 301-309.

Sommer, T.R., Potts.W.T. and Morrissy, N.M. (1990) Recent progress in the use of processed microalgae in aquaculture. *Hydrobiologia* 204(205): 435-443.

Sorgeloos, P., Dhert, P. and Candreva, P. (2001) Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture* 200: 147-159.

Sorokin, C. and Krauss, R.W. (1965) The dependence of the cell division in chlorella on temperature and light intensity. *American Journal of Botany* 52: 331-339.

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006) Commercial Applications of Microalgae. *Journal of Bioscience and Bioengineering* 101(2): 87–96.

Steel, J.A. (1980) Phytoplankton Models: Function of Fresh-water Ecosystems. pp 20-227. Cambridge University Press. Cambridge, UK.

Strain, H.H., and Svec, W.A. (1966). Extraction, separation, estimation and isolation of chlorophylls. The chlorophylls. *Academic* pp 21-66.

Strickland, J.D.H. and Parsons, T.R. (1968) A practical handbook of seawater analysis. *Bulletin Fish Research Canada* 167-311.

Striebel, M., Behl, S. and Stibor, H. (2009) The coupling of biodiversity and productivity in phytoplankton communities: consequences for biomass stoichiometry. *Ecology* 90: 2025–31.

Sunda, W.G., Price, N.M. and Morel, F.M.M. (2005) Trace metal ion buffers and their use in culture studies In Algal Culturing Techniques. pp 35-64. Elsevier Academic Press.

Sutherland, D.L., Turnbull, M.H., Broady, P.A. and Craggs, R.J. (2014) Effects of two different nutrient loads on microalgal production, nutrient removal and photosynthetic efficiency in pilot-scale wastewater high rate algal ponds. *Water Research* 66: 53-62.

Swarnalatha, G.V., Namratha, S.H., Vikas, S.C. and Sarada, R. (2015) The effect of carbon dioxide rich environment on carbonic anhydrase activity, growth and metabolite production in indigenous freshwater microalgae. *Algal Research* 9: 151–159.

Takagi, M. Karseno, and Yoshida, T. (2006) Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae Dunaliella cells. *Bioscience Bioengineering* 101:223–226.

Talling, J.F. (1969) General outline of spectrophotometric methods. *Aquatic Environments* 22-25.

Talling, J.F. (1981) The development of attenuance depth profiling to follow the changing distribution of phytoplankton and other particulate material in a productive English lake. *Arhive Hydrobiologia* 93: 1-20.

Tam, N.F.Y. and Wong, Y.S. (1996) Effect of ammonia concentratons on growth of Chlorella vulgaris and nitrogen removal from media. *Bioresource Technology* 57: 45-50.

Tamelen, P.G.V. (1978) Early succesional mechanisms in rocky interdal: The role of direct and indirect interactions. *Journal Experimental Marine Biology and Ecology* 112: 39-48.

Thompson, P.A., Bonhan, P.L., Swadling, P.I. and Swadling, K.M. (2008) Phytoplankton bloom in the Huon Estuary, Tasmania: Top-down or bottom-up control. *Journal Planktonic Research* 30: 735-753.

Torzillo, G., Accolla, P., Pinzani, E. and Masojidek, J. (1996) *In situ* monitoring of chlorophyll fluorescence to assess the synergistic effect of low temperature and high irradiance stress in *Spirulina* cultures grown outdoors in photobioreactors. *Journal Applied Phycology* 8: 283–291

Townsend, D.W., Keller, M.D., Sieracki, M.E. and Ackleson, S.G. (1992) Spring phytoplankton blooms in the absence of vertical water column stratification. *Nature* 360: 59-62.

Tredici, M.R. (2004) Mass production of microalgae: photobioreactors. Handbook of Microalgal Culture. *Biotechnology and Applied Phycology*. Blackwell Publishing. pp 178-214.

Trejo-Téllez L.I. and Gómez-Merino, F.C. (2012) Nutrient Solutions for Hydroponic Systems, Hydroponics: A Standard Methodology for Plant Biological Researches. Retrieved 21 June 2016 from http://cdn.intechopen.com/pdfs/33765.pdf.

Ugoala, E., Ndukwe, G., Mustapha, K.B. and Ayo, R.I. (2012) Constraints to large scale algae biomass production and utilization. *Journal of Algal Biomass Utilization* 3(2): 14- 32.

Ukeles, R. (1980) American experience in the mass culture of microalgae for feeding larvae of the american oyster *Crassostrea virginica. Algal Biomass* 35: 287-306.

United Nations (1996) Introduction to United Nation Convention to combat desertification. pp 56-93.

United Nations (2007) United Nation Framework Convention on Climate change. pp 84-134.

Valenzuela-Espinoza, E., Milan-Nunez, R. and Nunez-Cebrero, F. (2001) Protein, carbohydrate and chlorophyll a content in Isochrysis aff. Galbana (clone T-Iso) cultured with low cost alternative to the f/2 medium. *Aquacultural Engineering* 25: 207-216

Vandamme, D., Foubert, I., Meesschaert, B. and Muylaert, K. (2010) Flocculation of microalgae using cationic starch. *Journal of Applied Phycology* 22(4): 525-530.

Vasseur, C., Bougaran, G., Garnier, M., Hamelin, J., Leboulanger, C., Le Chevanton, M., Mostahir, B., Sialve, B., Steyer, J.P., and Fouilland, E. (2012) Carbon conversion efficiency and population dynamics of a marine algae-bacteria consortium growing on simplified synthetic digestate: first step in a bioprocess coupling algal production and anaerobic digestion. pp 79-87.

Venkata Mohan, S., Prathima Devi, M., Mohankrishna, G., Amarnath, N., Lenin Babu, M., and Sarma, P.N. (2011) Potential of mixed microalgae to harness biodiesel from ecological waterbodies with simultaneous treatment. *Bioresource Technology* 102: 1109-1117.

Vonshak, A. (1997) Outdoor mass production of *Spirulina*: The basic concept. In: Vonshak A (ed.), *Spirulina platensis (Arthrospira)*. *Physiology, Cell Biology and Biotechnology* pp 79–99. Taylor and Francis.

Wang, B., Li, Y., Wu, N. and Lan, C.Q. (2008) CO_2 bio-mitigation using microalgae. *Applied Microbiology and Biotechnology* 79(5): 707–718.

Wang, G., Chen, H., Li, G.,Chen, L., Li, D., Hu, G., Chen, K., and Liu, Y. (2006) Population growth and physiological characteristics of microalgae in a miniaturized bioreactor during space flight. *Acta Astronautica* 58: 264-269.

Weissman, J.C., Goebel, R.P., and Benemann, J.R. (1988) Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. *Biotechnology Bioengineering* 31: 336–344.

Whittaker, R.H. (1975) Communities and ecosystem, pp 61-65. Macmillan, New York, USA.

Wijffels, R.H. and Barbosa, M.J. (2010) An outlook on microalgae bioluels. *Science* 329: 796-799.

Wikfors, G. (1986) Altering growth and gross chemical composition of two microalgal molluscan food species by varying nitrate and phosphate. *Aquaculture* 59: 1–14.

World Health Organization (1999) Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. pp 416-417.

Wurts, W.A. (2010) Farming algal fuel. Economics challenge, and potential. *Global Aquaculture Advocate* 13: 71-73.

Wyatt, N.B., Gloe, L.M., Brady, P.V., Hewson, J.C., Grillet, A.M., Hankins, M.G. and Pohl, P.I. (2011) Critical conditions for ferric chloride-induced flocculation of freshwater algae. *Biotechnology and Bioengineering* 109: 493-501.

Xin, L., Hong-Ying, H., Ke, G., and Ying-Xue, S. (2010) effects of different nitrogen and phosphorus concentrations on growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. *Bioresource Technology* 101: 494-500.

Xin, W., Chunbo, H., Zhang, F., Feng, C. and Yang, Y. (2011) Inhibition of the growth of two blue-green algae species (*Microsystis aeroginosa* and *Anabaena spiroides*) by acidification treatments using carbon dioxide. *Bioresource Technology* 102(10): 5742-5748.

Yang, Z., Geng, L., Wang, W. and Zhang, J. (2012) Combined effects of temperature light intensity, and nitrogen concentration on the growth and polysaccharide content of *Microcystis aeruginosa* in batch culture. *Biochemistry and System Ecology* 41: 130–135.

Yilancioglu, K., Cokol, M., Pastirmaci, I., Erman, B. and Cetiner, S. (2014) Oxidative stress is a mediator for increased lipid accumulation in a newly isolated *Dunaliella salina* strain. *Public Library of Science One* 9(3): 919-957.

Yuen, S.H. and Pollard, A.G. (2006) Deniges' method for determination of phosphate with special reference to soil solutions and extracts. *Journal of the Science of Food and Agriculture* 6(4): 223-229.

Zhu, C.J., Lee, Y.K. and Chao, T.M. (1997) Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. *Journal of Applied Phycology* 9: 451–457.